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# In This Issue . . .

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## Requisition of Keratinocyte Proliferation . . .

Keratinocyte stem cells are thought to be among the more slowly proliferating basal cells located in proximity to epidermal Langerhans cells (ECLs). Based on this observation, Potten and Allen hypothesized nearly twenty years ago that ECLs might regulate these stem cells. Until now there has been no molecular evidence to support this idea. In this issue, Parkinson *et al* (p. 113) describe the tissue specificity of the newly identified hematopoietic stem cell inhibitor/macrophage inflammatory protein 1$\alpha$ (SCI/MIP-1$\alpha$). Their emphasis is placed on identification of cells that produce the cytokine as well as its influence on the proliferative response of keratinocytes. This study provides the first evidence that "ECLs regulate the keratinocyte cell cycle by the production of an inhibitor of keratinocyte proliferation."

SCI/MIP-1$\alpha$ is a potent reversible inhibitor of keratinocyte proliferation as measured by its effect on plating and cloning efficiencies as well as on growth rates of cultured human and mouse keratinocytes. It is not, however, produced by the keratinocytes themselves as are other keratinocyte proliferation inhibitors such as transforming growth factor $\beta$, tumor necrosis factor, and interferon. The only epidermal cell type to contain detectable SCI/MIP-1$\alpha$ transcripts is ECLs, which are hematopoietic in origin. Thus, SCI/MIP-1$\alpha$ is the first cytokine shown to operate by a paracrine mechanism within the skin. This group proposes a mechanism for control of epidermal cell proliferation in which "the ECL acts as the source of a potent reversible inhibitory molecule that is produced in the locale of the stem cells and may be maintained in this restricted area by virtue of its tendency to bind proteoglycan residues." SCI/MIP-1$\alpha$ could be involved *in vivo* in keratinocyte homeostasis as well as in hyperproliferation in wound repair, psoriasis, cancer, and other skin disorders. Furthermore, interleukin-8, a closely related chemokine, stimulates epidermal cell proliferation and may act to balance the effects of the inhibitor.

## . . . And Keratinocyte Differentiation.

Along their maturation pathway from the basal layer to the stratum corneum, epidermal keratinocytes undergo a series of morphologic and biochemical changes. One of the later events, in cells of the granular layer and lower stratum corneum, is the appearance of keratohyalin granules resulting from transcription and production of a new protein, profilaggrin. This large precursor contains many repeats of filaggrin, which causes aggregation of intermediate filaments *in vitro* and is thought to be involved in alignment of keratin bundles for interfilament disulfide cross-linking *in vivo*. Later, in the upper stratum corneum, filaggrin is broken down to component amino acids, which may help to bind water molecules in the upper stratum corneum. Cultured keratinocytes express many of the biochemical markers of epidermal differentiation, including profilaggrin, once the cells reach confluence and overall protein synthesis decreases.

Ichthyosis vulgaris is an autosomal dominant inherited scaling disorder of the skin. Keratohyalin granules, profilaggrin, and filaggrin are reduced or absent in biopsies or keratinocyte cultures from affected individuals. In one model of this disorder, reduced levels of filaggrin cause decreased water retention by amino acids in the stratum corneum, and "dry" skin results. Haydock and co-workers (p. 118) present a study in this issue that tests this hypothesis by using "anti-sense technology" to inhibit profilaggrin production and produce the ichthyosis vulgaris phenotype in cultured keratinocytes. Transformation with a plasmid that produces an RNA transcript complementary to profilaggrin mRNA causes inactivation of the message and inhibition of the synthesis of profilaggrin. As predicted, keratohyalin granules were reduced and cell keratinocyte morphology was abnormal. However, other morphologic and biochemical disturbances, including a delay in the decrease of protein synthesis associated with confluence, were found. These results suggest an additional, more global effect of profilaggrin on *in vitro* epidermal differentiation and demonstrate the utility of anti-sense technology in keratinocyte biology.

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## Anti-Sense Tips the Scales of Psoriasis Toward Macrophages

The most obvious clinical and histologic aspects of psoriasis are 1) an increase in the number of proliferating cells in the basal epidermis, which leads to scaly, plaque-like lesions, and 2) an inflammatory infiltrate that causes their erythematous appearance. The presence of infiltrating lymphocytes has suggested the involvement of immunologic processes in the pathogenesis of psoriasis. Lymphocytes and neutrophils that immigrate into the epidermis have been the focus of most studies. In this issue Gillitzer *et al* (p. 127) examine a population of potentially mobile population of CD68+ macrophages and demonstrate that the "dermal macrophages are accumulated in the subepidermal space . . . often aligned along the tips of elongated rete ridges like pearls." They asked, "What are the soluble signals that contribute to this restricted distribution of dermal macrophages?" and "Is there a functional interrelationship between dermal macrophages and the highly proliferative basal keratinocytes in psoriasis?" *In situ* hybridization of anti-sense $^{35}$S-RNA probes for the potent monocyte chemotactic protein-1 (MCP-1) detected mRNA in scattered cells within the subepidermal space and "most surprisingly, in the basal keratinocytes of the tips of the rete ridges of psoriatic lesions. Thereby, every keratinocyte in a row expresses high levels of MCP-1 [and] a specific gradient is created between the basal layer and the dermal compartment." Dermal macrophages may recognize this gradient. The restricted location of MCP-1 correlates with high rate of proliferation in the keratinocytes expressing MCP-1 message and with the location of macrophages on the dermal side of the basement membrane near the rete pegs. The authors suggest "there is a dialogue between proliferating basal keratinocytes and dermal macrophages with MCP-1 as one of the communicating signals."

## Becoming Sensitive to Hapten Dosage

Since the early 1970s most cutaneous antigen presenting function has been attributed to epidermal Langerhans cells. Recently, B. Nickoloff, J.W. Streilein, and K. Cooper (among others) suggested that non-Langerhans *dermal* cells derived from the bone marrow also may function as antigen-presenting cells (APC), particularly after exposure of the skin to ultraviolet B (UVB) radiation. The question may hinge in part on the amount of hapten applied to the skin to sensitize the mice. In this issue, Kurimoto and Streilein (p. 132) have determined a so-called "optimized sensitizing dose" of dinitrofluorobenzene for UVB-susceptible and UVB-resistant strains of mice. Their results indicate that when conventional (high) sensitizing doses of hapten were used, both epidermal and dermal antigen presenting cells participated in the induction of contact hypersensitivity. However, with optimal epicutaneous sensitizing doses of antigen, only epidermal Langerhans cells contributed to the response.

When mouse skin was irradiated with UVB sufficient to obliterate epidermal Langerhans cells, conventional doses of hapten induced sensitivity in UVB-resistant but not UVB-susceptible strains, probably because dermal APC were missing or nonfunctional in the UVB-susceptible mice. In contrast, with the optimal sensitizing dose the authors were not able to sensitize by the epicutaneous route either UVB-resistant or UVB-susceptible strains of mice. Intracutaneous sensitization with this same optimal dose of hapten, however, produced sensitization in the UVB-resistant but not in the UVB-susceptible strains, demonstrating that the APC in the dermis remained functional in the UVB-resistant but not in the UVB-susceptible mice. At a lower dose of UV that altered morphology but did not eliminate Langerhans cells, epicutaneous application produced sensitivity in UVB-resistant but not in UVB-susceptible strains.

These results imply that the dose and means of delivery of the sensitizing hapten determine whether cells of the epidermis or dermis (or both) provide antigen presenting function and that Langerhans cells are not necessary for intracutaneous sensitization. Furthermore, since little UVB actually penetrated the dermis, and since UVB-irradiated mice still provide APC function, failure of the dermal response in UVB-susceptible mice "suggests that factors released from UVB-damaged epidermis diffuse into the dermis and suppress local APC function." Tumor necrosis factor $\alpha$, *cis*-urocanic acid, and DNA excision and repair enzymes are among the elements that may mediate this response.

## Hot Spots and Blistering

Some forms of epidermolysis bullosa are manifested in keratinocytes and involve abnormalities in the assembly of keratin filaments. The "simplex" class (EBS) is the mildest form and shows characteristic blistering in the epidermis, healing without scarring, basal cell cytolysis, and autosomal dominant inheritance of alterations in the keratin 5 and keratin 14 genes. Sporadic cases of EBS have also been diagnosed. The severity of the disorder may be related to the site of mutation within the keratin protein.

Some regions of genes are more likely to undergo mutation than others, so that many mutations may be found to be localized to these regions, which are called "hot spots". A major goal in understanding the molecular basis of any genetic disorder is a correlation between a particular mutation, the structure of the affected gene product, and the severity of the disorder. In this issue, Stephens *et al* (p. 240) report the occurrence of a hot spot in codon 125 of keratin 14 with genetic linkage to the Dowling-Meara subtype of EBS. This variant of EBA displays distinctive clumps of keratin filaments by electron microscopy. This is an important mutation because there may be extensive involvement of affected individuals at birth, and affected infants may die. Since 50% of the cases of EBS-DM studied carried mutations in codon 125, the authors recommend DNA-based prenatal diagnosis in families with EBS-DM. The reason for the high level of mutability of this region is unknown, but now that the hot spot has been identified, this question may be asked.